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14. ABSTRACT Abstract These studies are aimed at targeting a population of luminal epithelial cells that repopulate the prostate gland after androgen-ablation and give rise to castrate-resistant disease prostate cancer. In two specific aims, we proposed to first identify novel antigenic targets on these castrate resistant luminal epithelial cells (CRLEC), then to test these antigens with state-of-the-art vaccines. During the first year of funding we've made significant progress on Aim 1, and microarray studies to identify antigenic targets are in progress at the JHU core facility. Our second model, involving implanted tumor cells in mice, was a bit more challenging and we were unable to sort CRLEC with standard technology. Instead, we had to work around that issue by generating a fluorescently tagged cell line (mCherry-MycCap). That cell line has been established and characterized, and the additional CRLEC antigen studies that are part of Aim 1 are currently making good progress.					
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1) Introduction: The goal of this project is to target a population of luminal epithelial cells that repopulate the prostate gland (and cancer) after androgen-ablation and give rise to castrate-resistant disease. These cells, which we have termed Castrate-Resistant Luminal Epithelial Cells (CRLEC) represent a relatively rare population, encompassing approximately 5% of the total epithelial cells in the gland(1). Destruction of CRLEC will be performed immunologically, as the normal adult mammalian immune system is exquisitely capable of destroying a targeted cell population, often without considerable bystander damage to adjacent cells(2). In addition to standard microarray studies, which are technically feasible and straightforward, we are employing a more innovative approach in which we ask the immune system to decide which CRLEC targets it deems most interesting. To accomplish that task, we plan to use CRLEC as one component of a cell-based vaccine, and profile the antibody response generated using advanced serological analysis approaches(3-5). To our knowledge, this concept has yet to be applied to prostate cancer. Our two distinct approaches to identification of CRLEC targets overlap – by employing multiple approaches we hope to generate a more robust set of targets than if a single method were to be employed. In the later years of this grant, we will screen a subset of these targets, using a novel vaccine platform based on the recently discovered adjuvant known collectively as cyclic dinucleotides (CDN). Using this adjuvant permits us to generate robust immune responses using whole protein-based vaccines, and thus enables rapid screening of a number of promising CRLEC targets. Finally, we will evaluate promising vaccines pre-clinically using well-accepted immuno-competent animal models.

2) Keywords: Prostate Cancer, Lymphocyte, Vaccine, Antibody

3) Overall Project Summary

Specific Aims

1) Identify and verify antigenic targets specifically associated with CAstrate Resistant Luminal Epithelial Cells (CRLEC's).

2) Using a novel vaccine platform based on cyclic di-nucleotides (CDN) as an adjuvant, rapidly screen a panel of promising CRLEC targets in intact and castrate animals.

Task 1: Identify and verify antigenic targets from CAstrate Resistant Luminal Epithelial Cells (CRLEC)

(months 1-16).

Subtask 1a1:

Isolate and transcriptionally profile Castrate-Resistant Luminal Epithelial Cells from intact HoxB13-GFP mice. (months 1-8).

Description:

CRLEC from micro-dissected single cell prostate suspensions from castrate HOXB13-GFP mice will be isolated using flow sorting. Approximately 5 animals per group will be processed to derive sufficient RNA for microarray analysis; the experiment will be repeated x 3. Microarray analysis will be performed in collaboration with the Johns Hopkins microarray core.

This task has been accomplished, GFP positive cells from both intact and castrated HOXB13-GFP mice were sorted to > 95% purity. Sufficient cell numbers (> 5000) were obtained in three separate experiments in biological repeats. RNA was extracted from these cells and samples are currently under analysis in the JHU imcroarray core. This Aim is approximately 4 months behind schedule due to unexpectedly slow animal breeding at the University of Maryland. We are currently in the process of transferring the animals to JHU in case respeat studies are required.

Subtask 1a2:

Isolate and profile transcriptionally profile Castrate-Resistant Luminal Cancer Cells from tumors extracted from mice bearing Myc-CaP tumors post-castration (months 1-8).

Description:

Castrate-resistant Myc-CaP tumor cells from implanted tumors will be sorted on day + 7 post castration. Approximately 5 animals per group will be processed to derive sufficient RNA for microarray analysis; the experiment will be repeated x 3. Microarray analysis will be performed in collaboration with the Johns Hopkins microarray core.

This task proved intractable, despite using a variety of markers we were unable to identify a series of cell surface markers capable of specifically sorting castration-resistant epithelial cells from implanted MycCaP tumors away from the stroma. To circumvent this issue, we adopted an alternative approach, hypothesizing that a bright fluroescent marker like mCherry would allow more facile identification and sorting of CRLEC from MycCaP. This proved successful, as shown in Figure 1 we now have series of mCherry-MycCaP cell lines that are 1) Stable 2) grow normally in immunocompetent hosts 3) are easily sorted. These cells are currently growing in immunocompetent wildtype FVB/n mice. Sorting experiments are planned for Q4 2014, and these studies will be back on track by early 2015.

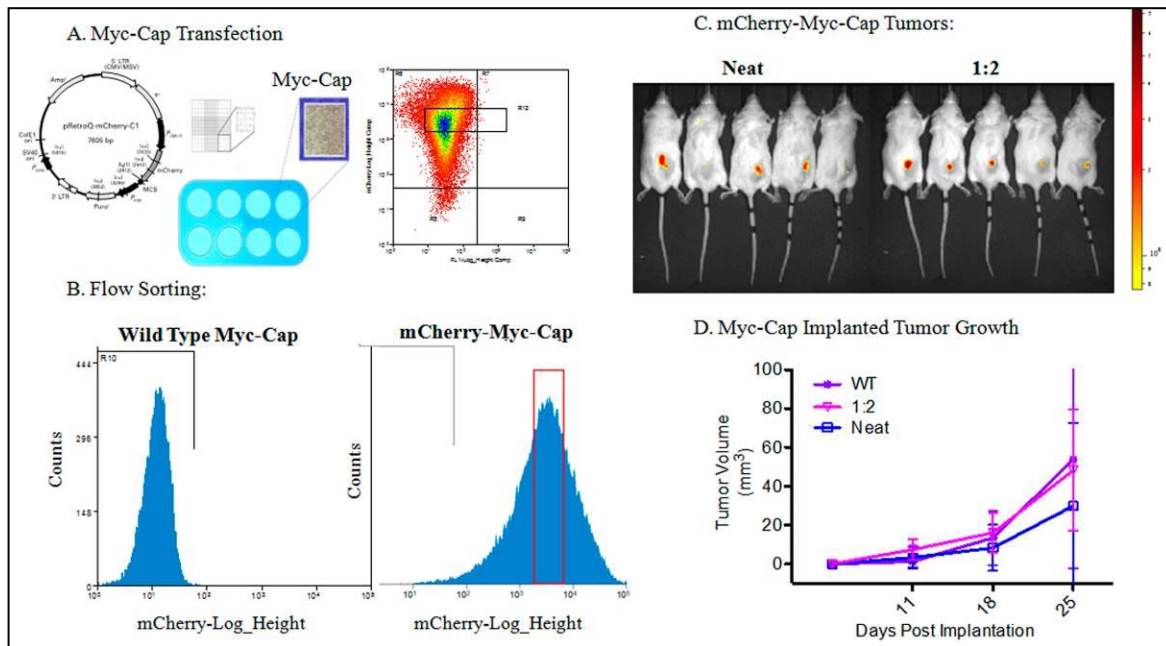


Figure 1: Myc-Cap and mCherryMyc-Cap Tumorogenesis Capacity in In-Vivo Tumor Model.

A. Myc-Cap cells were plated and transfected with pRetroQ-mCherry-C1 using two different titrations of lipofectamine (27.1ul and 43.4ul) and vector (neat and 1:2 dilution), then selected on

puromycin to establish mCherryMyc-Cap cells. Flow cytometry confirmed robust fluoro-chrome expression. B. mCherryMyc-Cap cells, expressing intermediate levels of mCherry, were isolated by flow sorting and expanded *in vitro*. C. Sorted 27.1 neat and 1:2 mCherryMyc-Cap tumors 18 days post implantation. D. Sorted mCherryMyc-Cap cell and Myc-Cap wild type cells were implanted into male FBV^b mice and tumor growth was measured over time.

Subtask 1b:

Determine the serological profile of Castrate-Resistant Luminal Epithelial Cells (Months 6-12).

Description:

GFP positive, castration resistant epithelial cells will be flow sorted from the prostate glands of castrate HOXB13-GFP transgenic mice, and used to vaccinate female WT mice. Sera from prime / boosted mice will be collected and high-throughput protein array screens performed. Bioinformatics analyses will be performed to identify CRLEC antibody targets.

This task has been initiated, but was slightly delayed by the relative paucity of HOX-B13 GFP mice available to us. Nevertheless, we have now vaccinated WT mice with sorted castration resistant luminal epithelial cells from both HOX-B13 GFP animals. Serological studies will be completed in Q1 or Q2 of 2015.

Subtask 1c:

Generate and verify a prioritized list of CRLEC targets for further study (Months 10-16).

Description:

Genes over-expressed in castrate-resistant luminal epithelial cells versus their castrate-sensitive counterparts, genes over-expressed in castrate-resistant luminal tumor cells (Myc-CaP) versus their castrate-sensitive counterparts, and protein targets recognized by the immune system upon pro-immunogenic encounter with CRLEC's. will be integrated to form a prioritized list of 5-15 protein targets for further interrogation. Targets expression will be verified using qPCR, flow-cytometry or Western blotting analyses.

This task is pending.

4) Key Research Accomplishments:

- CRLEC from HoxB13 GFP mice have been sorted, RNA extracted, and samples submitted to the microarray core for analysis.
- A mCherry-MycCAP cell line has been successfully generated to address issues sorting epithelial cells from implanted MycCap tumors.
- Vaccination studies have been initiated, and sera will be harvested after boost vaccination.

5) Conclusion

Progress during the first year of this project was substantial, although slightly less than that outlined by our work plan. This was due to 3 issues: 1) A relative paucity of HoxB13-GFP mice. This has been overcome by increased breeding efforts by our collaborators at the University of Maryland. In addition we are transferring these mice to the JHU breeding facility so that we can more closely match breeding and animal generation to our current needs. 2) Challenges in sorting epithelial cells from MycCap tumors. This challenge was nicely overcome by the generation of mCherry-MycCaP

cells, these cells have proven easy to grow and sort and are allowing us to make rapid progress on Aim 1. 3) Loss of key personnel – The graduate student assigned to this project has left the graduate program for an alternative career. A new graduate student with considerable technical experience (and drive) has assumed control and is making rapid progress. Hence we are confident that by the end of the next reporting period (year 2) we will have completed all the tasks originally planned for the first 2 years of the project and will have made substantial progress toward the goal of generating a novel prostate cancer vaccine aimed at targeting the castration resistant epithelial cells left behind after initial androgen ablation.

6) Publications, Abstracts, and Presentations

Alme A, Drake CG. (2013) Immunological Targeting of Castrate Resistant Luminal Epithelial Cells (CRLECs). Johns Hopkins Tumor Immunology Research in Progress Seminar, Baltimore, MD, October 14, 2013.

7) Inventions, Patents and Licenses

Nothing to report

8) Reportable Outcomes

- Graduate Student Angela Alme was taken off this project and was replaced by Ms. Zoila Areli Lopez Bujanda, who will assume control of the proposed studies going forward.

9) Other Achievements

Nothing to report

10) References

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